1: Crit Rev Ther Drug Carrier Syst. 2002;19(3):191-233. Related Articles, Links

Factors affecting drug and gene delivery: effects of interaction with blood components.

Opanasopit P, Nishikawa M, Hashida M.

Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

Targeted drug delivery systems have been used extensively to improve the pharmacological and therapeutic activities of a wide variety of drugs and genes. In this article, we summarize the factors determining the tissue disposition of delivery systems: the physicochemical and biological characteristics of the delivery system and the anatomic and physiological characteristics of the tissues. There are several modes of drug and gene targeting, ranging from passive to active targeting, and each of these can be achieved by optimizing the design of the delivery system to suit a specific aim. After entering the systemic circulation, either by an intravascular injection or through absorption from an administration site, however, a delivery system encounters a variety of blood components, including blood cells and a range of serum proteins.These components are by no means inert as far as interaction with the delivery system is concerned, and they can sometimes markedly effect its tissue disposition. The interaction with blood components is known to occur with particulate delivery systems, such as liposomes, or with cationic charge-mediated delivery systems for genes. In addition to these rather nonspecific ones, interactions via the targeting ligand of the delivery system can occur. We recently found that mannosylated carriers interact with serum mannan binding protein, greatly altering their tissue disposition in a number of ways that depend on the properties of the carriers involved.

PMID: 12627613 [PubMed - in process]

1: J Control Release. 1998 Apr 30;53(1-3):25-37. Related Articles, Links

Targetable HPMA copolymer-adriamycin conjugates. Recognition, internalization, and subcellular fate.

Omelyanenko V, Kopeckova P, Gentry C, Kopecek J.

Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City 84112, USA.

Recognition, internalization, and subcellular trafficking of N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer conjugates containing N-acylated galactosamine (GalN) or monoclonal OV-TL16 antibodies (Ab) have been investigated in human hepatocarcinoma HepG2 and ovarian carcinoma OVCAR-3 cells, respectively. The intrinsic fluorescence of fluorescein or adriamycin (ADR) attached to HPMA copolymers permitted us to follow the subcellular fate of HPMA copolymer conjugates by confocal fluorescence microscopy and fluorescence spectroscopy. The pattern of fluorescence during incubation of HPMA copolymer-ADR-GalN conjugate containing lysosomally degradable tetrapeptide (GFLG) side-chains with HepG2 cells was consistent with conjugate recognition, internalization, localization in lysosomes, followed by the release of ADR from the polymer chains and ultimately diffusion via the cytoplasm into the cell nuclei. A similar pattern was observed in OVCAR-3 cells for Ab targeted HPMA copolymer conjugates. To test our hypothesis that HPMA-copolymer-bound anticancer drugs will be inaccessible to the energy-driven P-glycoprotein efflux pump in multidrug resistant (MDR) cells, we have compared the internalization of the HPMA copolymer-ADR conjugates by sensitive (A2780) and ADR-resistant (A2780/AD) ovarian carcinoma cell lines. Preliminary data on relative retention of ADR in MDR (A2780/AD) cells indicate a higher intracellular ADR concentration after incubation with HPMA copolymer-ADR conjugate when compared to incubation with free (unbound) ADR.

PMID: 9741911 [PubMed - indexed for MEDLINE]

1: J Biochem (Tokyo). 1999 Nov;126(5):879-88.

Related Articles, Links

JB-online

Entrez PubMed

Relationship between the subcellular localization and structures of catalytic domains of FKBP-type PPIases.

Himukai R, Kuzuhara T, Horikoshi M.

Laboratory of Developmental Biology, Department of Cellular Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, 113-0032, Japan.

PubMed Services

The Schizosaccharomyces pombe gene, fkp39(+), encoding a homolog of FKBP(FK506 binding protein)-type peptidyl prolyl cis-trans isomerase (PPIase), was isolated and the primary structure was determined. This gene product (SpFkbp39p) showed PPIase enzymatic activity in a chymotrypsin-dependent enzyme assay involving recombinant SpFkbp39p. Comparison of the primary structures of the catalytic domains of FKBPs, including SpFkbp39p, revealed that FKBPs could be classified into four groups. This categorization corresponding to the known subcellular localization of the FKBPs, makes the prediction of the subcellular localization of FKBPs based on their primary structures feasible. SpFkbp39p was considered to be a member of the nuclear-type FKBP group from this relationship between primary structure and subcellular localization. An immunofluorescence assay against HA-epitope-tagged SpFkbp39p revealed that SpFkbp39p is localized to the nucleus, as predicted. Residues conserved in a "group-specific" manner in the catalytic domain were mapped to their corresponding three-dimensional positions; these "group-specific" residues were located in close proximity in distinct regions mostly on the protein surface, which implies the presence of "group-specific" regulatory functional regions. We also found that nuclear-type FKBPs, including SpFkbp39p, have two highly conserved domains other than catalytic ones, with further basic and acidic charged regions, especially in the case of nuclear-type FKBPs. This is the first report indicating that there is a rule for the relationship between the subcellular localization and structure of the catalytic domain of a FKBP.

PMID: 10544281 [PubMed - indexed for MEDLINE]

Related Resources

1: Belhadj-Tahar H, Ouhayoun E, Cros G, Darbieu MH, Tafani JA, Fabre J, Esquerre JP, Coulais Y. Related Articles

Synthesis and biodistribution of new oxo and nitrido 99mTc complexes with asymmetrical potentially dianionic or trianionic tetradentate SNNO ligands derived from methyl-2-aminocyclopentene-1-dithiocarboxylic acid.
Nucl Med Biol. 1998 Jan;25(1):65-9.
PMID: 9466364 [PubMed - indexed for MEDLINE]

2: Williams LE, Lewis MR, Bebb GG, Clarke KG, Odom-Maryon TL, Shively JE, Raubitschek AA. Related Articles

Biodistribution of 111In- and 90Y-labeled DOTA and maleimidocysteineamido-DOTA conjugated to chimeric anticarcinoembryonic antigen antibody in xenograft-bearing nude mice: comparison of stable and chemically labile linker systems.
Bioconjug Chem. 1998 Jan-Feb;9(1):87-93.
PMID: 9460550 [PubMed - indexed for MEDLINE]

3: Deal KA, Motekaitis RJ, Martell AE, Welch MJ. Related Articles

Evaluation of the stability and animal biodistribution of gadolinium (III) benzylamine-derivatized diethylenetriaminepentaacetic acid.
J Med Chem. 1996 Aug 2;39(16):3096-3106.
PMID: 8759630 [PubMed - indexed for MEDLINE]

4: Meijs WE, Haisma HJ, Van der Schors R, Wijbrandts R, Van den Oever K, Klok RP, Pinedo HM, Herscheid JD. Related Articles

A facile method for the labeling of proteins with zirconium isotopes.
Nucl Med Biol. 1996 May;23(4):439-48.
PMID: 8832698 [PubMed - indexed for MEDLINE]

5: Baxter LT, Jain RK. Related Articles

Pharmacokinetic analysis of the microscopic distribution of enzyme-conjugated antibodies and prodrugs: comparison with experimental data.
Br J Cancer. 1996 Feb;73(4):447-56.
PMID: 8595158 [PubMed - indexed for MEDLINE]

6: Jones-Wilson TM, Motekaitis RJ, Sun Y, Anderson CJ, Martell AE, Welch MJ. Related Articles

New hydroxybenzyl and hydroxypyridylmethyl substituted triazacyclononane ligands for use with gallium(III) and indium(III).
Nucl Med Biol. 1995 Oct;22(7):859-68.
PMID: 8547883 [PubMed - indexed for MEDLINE]

7: Stalteri MA, Mather SJ. Related Articles

A cross-linked monoclonal antibody fragment for improved tumor targeting.
Bioconjug Chem. 1995 Mar-Apr;6(2):179-86.
PMID: 7599261 [PubMed - indexed for MEDLINE]

8: Anderson CJ, John CS, Li YJ, Hancock RD, McCarthy TJ, Martell AE, Welch MJ. Related Articles

N,N'-ethylene-di-L-cysteine (EC) complexes of Ga(III) and In(III): molecular modeling, thermodynamic stability and in vivo studies.
Nucl Med Biol. 1995 Feb;22(2):165-73.
PMID: 7767309 [PubMed - indexed for MEDLINE]

☐ **9:** Kuus-Reichel K, Knott C, Sam-Fong P, Petrella E, Corvalan JR. Related Articles

Therapy of streptozotocin induced diabetes with a bifunctional antibody that delivers vinca alkaloids to IL-2 receptor positive cells.
Autoimmunity. 1995;22(3):173-81.
PMID: 8734571 [PubMed - indexed for MEDLINE]

☐ **10:** Sun B. Related Articles

[Comparative studies of 111In-labeled monoclonal antibody using spacer-containing and non-spacer bifunctional chelates: (I). Conjugation, labeling, immunoreactivity and in vitro stability].
Kaku Igaku. 1994 May;31(5):459-71. Japanese.
PMID: 8028218 [PubMed - indexed for MEDLINE]

☐ **11:** Baidoo KE, Lever SZ, Scheffel U. Related Articles

Bifunctional chelator for facile preparation of neutral technetium complexes.
Bioconjug Chem. 1994 Mar-Apr;5(2):114-8.
PMID: 8031873 [PubMed - indexed for MEDLINE]

☐ **12:** Morelli D, Menard S, Pozzi B, Balsari A, Colnaghi MI. Related Articles

Effect of a bifunctional monoclonal antibody directed against a tumor marker and doxorubicin on the growth of epidermoid vulvar carcinoma grafted in athymic mice.
Cell Biophys. 1994;24-25:119-26.
PMID: 7736515 [PubMed - indexed for MEDLINE]

☐ **13:** Anderson CJ, Rocque PA, Weinheimer CJ, Welch MJ. Related Articles

Evaluation of copper-labeled bifunctional chelate-albumin conjugates for blood pool imaging.
Nucl Med Biol. 1993 May;20(4):461-7.
PMID: 8504288 [PubMed - indexed for MEDLINE]

☐ **14:** Smith-Jones PM, Fridrich R, Kaden TA, Novak-Hofer I, Siebold K, Tschudin D, Maecke HR. Related Articles

Antibody labeling with copper-67 using the bifunctional macrocycle 4-[(1,4,8,11-tetraazacyclotetradec-1-yl)methyl]benzoic acid.
Bioconjug Chem. 1991 Nov-Dec;2(6):415-21.
PMID: 1805938 [PubMed - indexed for MEDLINE]

☐ **15:** Kostenikov NA, Fadeev NP, Serzhanina VA, Korsakova LN, Rozenberg OA. Related Articles

[An experimental study of tumor-tropic properties of radionuclide-labeled liposomes].
Med Radiol (Mosk). 1991;36(9):39-41. Russian.
PMID: 1943584 [PubMed - indexed for MEDLINE]

☐ **16:** Arano Y, Yokoyama A, Furukawa T, Horiuchi K, Yahata T, Saji H, Sakahara H, Nakashima T, Koizumi M, Endo K, et al. Related Articles

Technetium-99m-labeled monoclonal antibody with preserved immunoreactivity and high in vivo stability.
J Nucl Med. 1987 Jun;28(6):1027-33.
PMID: 3585492 [PubMed - indexed for MEDLINE]

☐ **17:** Vera DR, Stadalnik RC, Krohn KA. Related Articles

Technetium-99m galactosyl-neoglycoalbumin: preparation and preclinical studies.
J Nucl Med. 1985 Oct;26(10):1157-67.
PMID: 4045560 [PubMed - indexed for MEDLINE]

1: Pharm Res. 1997 Dec;14(12):1759-64. Related Articles, Links

Cellular delivery of oligonucleotides by synthetic import peptide carrier.

Dokka S, Toledo-Velasquez D, Shi X, Wang L, Rojanasakul Y.

Department of Basic Pharmaceutical Sciences, West Virginia University, School of Pharmacy, Morgantown, West Virginia 26506, USA.

PURPOSE: Inefficient cellular uptake and endosomal entrapment are among the obstacles impeding the therapeutic use of oligonucleotides (ONs). The objectives of this study are to investigate the feasibility of utilizing a synthetic import peptide as a drug carrier for cytoplasmic delivery of ONs and to study its transport mechanisms. METHODS: A molecular conjugate consisting of a signal import peptide (IP) derived from Kaposi fibroblast growth factor (K-FGF) and a polycationic ON linker, polylysine (PL), was synthesized and complexed with 5' fluorescently-labeled ON. Complex formation was verified by spectral shift assay and cellular uptake of the ON complex was studied fluorometrically. Microscopic studies were performed to visualize the intracellular distribution of the ON. RESULTS: Cells treated with the ON:IP-PL complex exhibited a dose-dependent increase in ON uptake over free ON-treated controls. The uptake of the complex was shown to occur via an energy-independent, non-endocytic, process since metabolic and endocytic inhibitors and low temperature did not prevent the uptake. Microscopic studies revealed a non-punctate fluorescence pattern, consistent with the non-endocytic transport process. Intense nuclear fluorescence was observed in cells treated with the complex but not with free ON, suggesting enhanced cytoplasmic delivery and nuclear accumulation of the ON by the conjugate. Efficient complex uptake was shown to require both the ON-binding moiety PL and the IP moiety. The delivery system was found to be non-toxic at the concentrations used. CONCLUSIONS: The peptide carrier was effective in promoting the cellular uptake of ON. The mechanism by which the peptide facilitates ON uptake appears to involve a direct translocation of ON via a non-endocytic process. The peptide carrier has the potential to overcome the problem of ON endosomal entrapment and degradation.

PMID: 9453065 [PubMed - indexed for MEDLINE]

L1 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:380753 HCAPLUS
DOCUMENT NUMBER: 134:361402
TITLE: **Bifunctional** inhibitor molecules, their use in the disruption of protein-protein interactions and therapeutic applications
INVENTOR(S): Crabtree, Gerald R.; Stankunas, Kryn; **Briesewitz, Roger**; Wandless, Thomas
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
INT. PATENT CLASSIF.:
MAIN: C12N009-99
SECONDARY: C12N009-00
CLASSIFICATION: 1-12 (Pharmacology)
Section cross-reference(s): 7, 9
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001036612	A1	20010525	WO 2000-US31695	20001117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-166675 P 19991119
ABSTRACT:

Bifunctional inhibitor mols. and methods for their use in the inhibition of protein-protein interactions are provided. The subject ***bifunctional*** inhibitor mols. are conjugates of a target protein ligand and a blocking protein ligand, where these two moieties are optionally joined by a linking group. In the subject methods, an effective amt. of the ***bifunctional*** inhibitor mol. is administered to a host in which the inhibition of a protein-protein interaction is desired. The ***bifunctional*** inhibitor mol. simultaneously binds to its corresponding target and blocking proteins to produce a tripartite complex that inhibits the target protein-protein interaction. The subject methods and compns. find use in a variety of applications, including therapeutic applications.

SUPPL. TERM: protein interaction **bifunctional** inhibitor therapeutic
INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(FKBP (FK 506-binding protein), fusion protein with NFAT; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)
INDEX TERM: Heat-shock proteins
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(HSP 90, blocking protein; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Transcription factors
ROLE: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(NFAT (nuclear factor, activated T-cell), inhibition of; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Drug design
Drug targeting
Molecular association
Protein engineering
(**bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Fusion proteins (chimeric proteins)
ROLE: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Ligands
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Proteins, general, biological studies
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Albumins, biological studies
Steroid receptors
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(blocking protein; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Proteins, specific or class
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cytoskeleton-assocd., blocking protein; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Biological transport
(import, inhibition of NFAT transcription factor nuclear translocation; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Immunosuppressants
(inhibition of NFAT transcription factor; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: Receptors
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(vitamin, blocking protein; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: 95076-93-0, Peptidyl-prolyl isomerase
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

INDEX TERM: 53123-88-9, Rapamycin 79217-60-0, Cyclosporin 104987-11-3, FK506
ROLE: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(inhibition of NFAT transcription factor; **bifunctional** inhibitor mols., their use in disruption of protein-protein interactions and therapeutic applications)

REFERENCE COUNT: 4

REFERENCE(S):

(1) Briesewitz, R; Proc Natl Acad Sci USA 1999, V96, P1953 HCAPLUS
(2) Clardy, J; Borrowing To Make Ends Meet Proc Natl Acad Sci USA 1999, V96, P1826 HCAPLUS
(3) Crabtree, G; Trends in Biochemical Sciences 1996, V21, P418 HCAPLUS
(4) The Board Of Trustees Of The Leland Stanford Junior University; WO 9418317 A1 1994 HCAPLUS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31695

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 9/99, 9/00
US CL :435/183, 184

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/183, 184

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Merck Index

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, MEDLINE
search terms: (author names, cites refs), bifunctional, inhibitor, FK-506, FKBP, cyclophilin, NFAT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/18317 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 18 August 1994, page 30, lines 15-33, page 35, lines 8-24, claims 24-30 and 34-39.	1-26
X	CRABTREE, G.R. Three-Part Inventions: Intracellular Signaling and Induced Proximity. Trends in Biochemical Sciences. November 1996, Vol. 21, pages 418-422, see whole document.	1-26
X	CLARDY, J. Borrowing To Make Ends Meet. Proc. Natl. Acad. Sci. USA. March 1999, Vol. 96, pag4es 1826-1827, see whole document.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
30 JANUARY 2001

Date of mailing of the international search report
26 FEB 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
HARRY GUTTMAN, PHD
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31695

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRIESEWITZ, R. et al. Affinity Modulation Of Small-Molecule Ligands By Borrowing Endogenous Protein Surfaces. Proc. Natl. Acad. Sci. USA. March 1999, Vol. 96, pages 1953-1958, see whole document.	1-26

WHAT IS CLAIMED IS:

1. A non-naturally occurring bifunctional inhibitor molecule of less than about 5000 daltons that inhibits a binding event between a first target protein and a second binding protein, said bifunctional inhibitor molecule consisting of:

a target protein ligand and a blocking protein ligand optionally joined by a linking group;

wherein said bifunctional inhibitor molecule is capable of simultaneously binding said target protein and said blocking protein in a manner sufficient to inhibit said binding event.

2. The bifunctional inhibitor molecule according to Claim 1, wherein said bifunctional inhibitor molecule comprises a linking group.

3. The bifunctional inhibitor molecule according to Claim 1, wherein said bifunctional inhibitor molecule binds to a site of said target protein that is also bound by said second binding protein.

4. The bifunctional inhibitor molecule according to Claim 1, wherein said bifunctional inhibitor molecule binds to a site of said target protein that is not bound by said second binding protein.

5. The bifunctional inhibitor molecule according to Claim 1, wherein said blocking protein is an extracellular protein.

6. The bifunctional inhibitor molecule according to Claim 1, wherein said blocking protein is an intracellular protein.

7. The bifunctional molecule according to Claim 6, wherein said blocking protein is a peptidyl prolyl isomerase.

8. A synthetic bifunctional inhibitor molecule of less than about 5000 daltons and capable of inhibiting a binding event between a first target protein and a second binding protein, wherein said bifunctional inhibitor molecule is of the formula:

Z-L-X

wherein:

X is target protein ligand;

L is a bond or a linking group; and

Z is different from X and is a blocking protein ligand;

wherein said bifunctional inhibitor molecule is capable of simultaneously binding to said target protein and said blocking protein in a manner sufficient to inhibit said binding event.

9. The bifunctional inhibitor molecule according to Claim 8, wherein X binds to a site of said target protein that is also bound by said second binding protein.

10. The bifunctional inhibitor molecule according to Claim 8, wherein X binds to a site of said target protein that is not bound by said second binding protein.

11. The bifunctional inhibitor molecule according to Claim 8, wherein X has a molecular weight of from about 50 to 2000 D.

12. The bifunctional inhibitor molecule according to Claim 8, wherein said target protein is an extracellular protein.

13. The bifunctional inhibitor molecule according to Claim 8, wherein said target protein is an intracellular protein.

14. The bifunctional inhibitor molecule according to Claim 13, wherein said blocking protein is a peptidyl prolyl isomerase.

15. The bifunctional inhibitor molecule according to Claim 8, wherein Z has substantially no pharmacologic activity apart from binding to a blocking protein.

16. A method for inhibiting a binding event between a first target protein and a second binding protein in a host, said method comprising:

administering to said host an effective amount of a bifunctional inhibitor molecule of less than about 5000 daltons consisting of a target protein ligand and a blocking protein ligand optionally joined by a linking group, wherein said bifunctional inhibitor molecule is capable of simultaneously binding said target protein and said blocking protein in a manner sufficient to inhibit said binding event;

whereby a tripartite complex comprising said bifunctional inhibitor molecule, said target protein and said blocking protein is produced that inhibits said binding event.

17. The method according to Claim 16, wherein said bifunctional inhibitor molecule comprises a linking group.

18. The method according to Claim 16, wherein said bifunctional inhibitor molecule binds to a site of said target protein that is also bound by said second binding protein.

19. The method according to Claim 16, wherein said bifunctional inhibitor molecule binds to a site of said target protein that is not bound by said second binding protein.

20. The method according to Claim 16, wherein said tripartite complex is produced intracellularly.

21. The method according to Claim 16, wherein said tripartite complex is produced extracellularly.

22. The method according to Claim 16, wherein said blocking protein is endogenous to said host.

23. The method according to Claim 22, wherein said blocking protein is selected from the group consisting of: peptidyl-prolyl isomerases, Hsp90, steroid hormone receptors, cytoskeletal proteins, albumin and vitamin receptors.

24. The method according to Claim 16, wherein said bifunctional inhibitor molecule is administered as a pharmaceutical preparation.

25. A pharmaceutical preparation comprising a bifunctional inhibitor molecule according to Claim 1.

26. A kit comprising the pharmaceutical preparation according to Claim 25 and instructions for use in a therapeutic method.

L1 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:380414 HCAPLUS
DOCUMENT NUMBER: 134:371812
TITLE: Targeted **bifunctional** molecules and therapies based thereon
INVENTOR(S): **Briesewitz, Roger**; Crabtree, Gerald R.; Wandless, Thomas
PATENT ASSIGNEE(S): Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
INT. PATENT CLASSIF.:
MAIN: A61K038-00
CLASSIFICATION: 63-6 (Pharmaceuticals)
Section cross-reference(s): 1
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001035978	A1	20010525	WO 2000-US31702	20001117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-166580 P 19991119
ABSTRACT:
Targeted **bifunctional** mols. and methods for their use are provided. The subject targeted **bifunctional** mols. are conjugates of a drug moiety and a targeting moiety, where these two moieties are optionally joined by a linking group. The **bifunctional** mols. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted **bifunctional** mols. find use in a variety of therapeutic applications. For example, a ***bifunctional*** mol. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this **bifunctional** mol. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

SUPPL. TERM: drug **bifunctional** conjugate targeting
INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(FKBP (FK 506-binding protein); targeted **bifunctional** mols. and therapies based thereon)
INDEX TERM: Albumins, biological studies
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(serum; targeted **bifunctional** mols. and therapies based thereon)
INDEX TERM: Drug targeting
(targeted **bifunctional** mols. and therapies based thereon)
INDEX TERM: 54-05-7D, Chloroquine, drug conjugates 83-89-6D, Quinacrine, drug conjugates 127-69-5D, Sulfisoxazole, drug conjugates 53123-88-9D, Rapamycin, drug conjugates

104987-11-3D, FK506, drug conjugates
ROLE: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(targeted **bifunctional** mols. and therapies based thereon)

REFERENCE COUNT: 4
REFERENCE(S):
(1) Amkraut; US 5620708 A 1997 HCAPLUS
(2) Pomato; US 5965106 A 1999 HCAPLUS
(3) Pouletty; US 5843440 A 1998 HCAPLUS
(4) Virtanen; US 5718915 A 1998 HCAPLUS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31702

CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/00
US CL :424133.1, 136.1, 173.1, 153.1, 181.1, 183.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424133.1, 136.1, 173.1, 153.1, 181.1, 183.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST
bifunctional, targeting, ligand

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,843,440 A (POULETTY et al.) 01 December 1998, see entire document.	1-38
Y	US 5,965,106 A (POMATO et al.) 12 October 1999, see entire document.	1-38
Y	US 5,718,915 A (VIRTANEN et al.) 17 February 1998, see entire document.	1-38
Y	US 5,620,708 A (AMKRAUT et al.) 15 April 1997, see entire document.	1-38

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JANUARY 2001	Date of mailing of the international search report 23 FEB 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Cybille Delacroix-Muirheid TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600 Telephone No. (703) 308-0[illegible]

Form PCT/ISA/210 (second sheet) (July 1998) ★

WHAT IS CLAIMED IS:

1. A non-naturally occurring bifunctional molecule of less than about 5000 daltons consisting of a drug moiety and a targeting moiety, wherein said drug moiety and said targeting moiety are optionally joined by a linking group and said bifunctional molecule exhibits a modulated biodistribution upon administration to a host as compared to a free drug control.

2. The bifunctional molecule according to Claim 1, wherein said bifunctional molecule comprises a linking group.

3. The bifunctional molecule according to Claim 1, wherein said bifunctional molecule does not include a linking group.

4. The bifunctional molecule according to Claim 1, wherein said bifunctional molecule exhibits increased efficacy upon administration to a host as compared to a free drug control.

5. The bifunctional molecule according to Claim 1, wherein said targeting moiety binds to a protein.

6. The bifunctional molecule according to Claim 4, wherein said protein that is bound by said targeting moiety is an extracellular protein.

7. The bifunctional molecule according to Claim 1, wherein said protein that is bound by said targeting moiety is an intracellular protein.

8. A targeted synthetic bifunctional molecule of less than about 5000 daltons of the formula:

Z-L-X

wherein:

X is a drug moiety;

L is a bond or a linking group; and

Z is a targeting ligand;

wherein X and Z are different and said drug moiety has a modulated biodistribution upon administration to a host as compared to a free drug control.

9. The bifunctional molecule according to Claim 8, wherein said bifunctional molecule exhibits increased efficacy upon administration to a host as compared to a free drug control.

10. The bifunctional molecule according to Claim 8, wherein said drug moiety has a molecular weight of from about 50 to 2000 D.

11. The bifunctional molecule according to Claim 8, wherein said drug moiety binds to a protein target.

12. The bifunctional molecule according to Claim 8, wherein said targeting moiety binds to an extracellular protein.

13. The bifunctional molecule according to Claim 8, wherein said targeting moiety binds to an intracellular protein.

14. The bifunctional molecule according to Claim 8, wherein said bifunctional molecule comprises a linking group.

15. The bifunctional molecule according to Claim 8, wherein said targeting moiety has substantially no pharmacologic activity apart from binding to an endogenous protein of said host.

16. A method for modulating the biodistribution of a drug upon administration to a host, said method comprising:

administering to said mammalian host an effective amount of a bifunctional molecule of less than about 5000 daltons consisting of said drug or an active derivative thereof and a targeting moiety optionally joined by a linking group, wherein said bifunctional molecule has a modulated biodistribution upon administration to said host as compared to a free drug control;

whereby said biodistribution of said drug upon administration to said host is modulated as compared to a free drug control.

17. The method according to Claim 16, wherein said bifunctional molecule exhibits enhanced efficacy upon administration to said host as compared to a free drug control.

18. The method according to Claim 16,, wherein said bifunctional molecule exhibits reduced toxicity upon administration to said host as compared to a free drug control.

19. The method according to Claim 16, wherein said targeting moiety binds to an intracellular protein.

20. The method according to Claim 16, wherein said targeting moiety binds to an extracellular protein.

21. The method according to Claim 16, wherein said drug target is a protein.

22. The method according to Claim 21, wherein said bifunctional molecule comprises a linking group.

23. The method according to Claim 21, wherein said bifunctional molecule is administered as a pharmaceutical preparation.

24. A method for targeting a drug to an intracellular site of a mammalian host, said method comprising:

administering to said mammalian host an effective amount of a bifunctional molecule comprising a drug moiety and a targeting moiety optionally joined by a linking group, wherein said drug and targeting moieties bind to intracellular proteins and said bifunctional molecule exhibits a modulated biodistribution upon administration to a mammalian host as compared to a free drug control;

whereby said drug is targeted to an intracellular site of a mammalian host.

25. The method according to Claim 24, wherein said bifunctional molecule comprises a linking group.

26. The method according to Claim 24, wherein said bifunctional molecule does not include a linking group.

27. A method for targeting a drug to an extracellular site of a mammalian host, said method comprising:

administering to said mammalian host an effective amount of a bifunctional molecule comprising a drug moiety and targeting moiety optionally joined by a linking group, wherein said drug and a targeting moieties bind to extracellular proteins and said bifunctional molecule exhibits a modulated biodistribution upon administration to a mammalian host as compared to a free drug control;

whereby said drug is targeted to an extracellular site of a mammalian host.

28. The method according to Claim 25, wherein said bifunctional molecule comprises a linking group.

29. The method according to Claim 25, wherein said bifunctional molecule does not include a linking group.

30. In a method of administering a drug to a host in need of said drug, the improvement comprising:

administering to said host an effective amount of a bifunctional molecule of less than about 5000 daltons consisting of said drug or a derivative thereof covalently linked, either directly or through an optional linking group, to a targeting moiety.

31. The method according to Claim 29, wherein said host is a mammalian host.

32. The method according to Claim 30, wherein said mammalian host is human.

33. The method according to Claim 30, wherein said drug is a small molecule.

34. The method according to Claim 30, wherein said targeting moiety binds to an endogenous biodistribution modulating protein.

35. The method according to Claim 34, wherein said endogenous biodistribution modulating protein is an extracellular protein.

36. The method according to Claim 34, wherein said endogenous biodistribution modulating protein is an intracellular protein.

37. A pharmaceutical preparation comprising a bifunctional molecule according to Claim 1.

38. A kit comprising the pharmaceutical preparation according to Claim 35 and instructions for use in a therapeutic method.

L1 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:380323 HCAPLUS
DOCUMENT NUMBER: 134:361342
TITLE: Drug conjugates with pharmacokinetic modulating moieties, and therapies based thereon
INVENTOR(S): **Briesewitz, Roger**; Crabtree, Gerald R.; Wandless, Thomas
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
INT. PATENT CLASSIF.:
MAIN: A01N061-00
SECONDARY: A61K038-00; A61K038-43; C12N011-00; C12N011-02; C07K001-00; C07K017-00; C07K017-02; C07K017-06
CLASSIFICATION: 1-2 (Pharmacology)
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001035748	A1	20010525	WO 2000-US31701	20001117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-166633 P 19991119
ABSTRACT:

Bifunctional mols. and methods for their use are provided. The ***bifunctional*** mols. of the invention are conjugates of a drug moiety and a pharmacokinetic modulating moiety, optionally joined by a linking group. The ***bifunctional*** mols. are further characterized in that they exhibit at least one modulated pharmacokinetic property upon administration to a host as compared to a free drug control. The subject **bifunctional** mols. find use in a variety of therapeutic applications.

SUPPL. TERM: drug conjugate pharmacokinetic modulating moiety therapeutic
INDEX TERM: Coupling agents
Drug delivery systems
Drugs
Pharmacokinetics
(drug conjugate with pharmacokinetic modulating moiety, and therapeutic use)
INDEX TERM: Albumins, biological studies
Proteins, general, biological studies
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(drug conjugate with pharmacokinetic modulating moiety, and therapeutic use)
INDEX TERM: Drug metabolism
Liver
(hepatic fist-pass metab.; drug conjugate with pharmacokinetic modulating moiety, and therapeutic use)
INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(ligand-binding; drug conjugate with pharmacokinetic

modulating moiety, and therapeutic use)

REFERENCE COUNT: 8

REFERENCE(S):
(1) Briesewitz; Proceedings of the National Academy of Sciences 1999, V96, P1953 HCAPLUS
(2) Brochu; Antimicrobial Agents and Chemotherapy 1992, V36(10), P2166 HCAPLUS
(3) Cemu, B; WO 9101743 A 1991 HCAPLUS
(4) Chakraborty; Chemistry and Biology 1995, V2(3), P157 HCAPLUS
(5) Crabtree; Elsevier Trends Journal 1996, P418 HCAPLUS
(6) Kramer; The Journal of Biological Chemistry 1992, V267(26), P18598 HCAPLUS
(7) Lussow; Transplantation 1996, V62(12), P1703 HCAPLUS
(8) Pouletty; US 5843440 A 1998 HCAPLUS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31701

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :514/1, 2; 424/94.1; 435/174, 177; 530/402, 810, 812

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/1, 2; 424/94.1; 435/174, 177; 530/402, 810, 812

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,843,440 A (POULETTY et al) 01 December 1998, see entire document.	1-50
Y	WO 91/01743 A (CEMU BIOTEKNIK) 21 February 1991, see entire document.	1-50
Y	BRIESEWITZ et al. Affinity Modulation of Small-Molecule Ligands by Borrowing Endogenous Protein Surfaces. Proceedings of the National Academy of Sciences USA. March 1999, Vol. 96, pages 1953-1958, see entire document.	1-50

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 FEBRUARY 2001	Date of mailing of the international search report 12 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID M. NAFF Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31701

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BROCHU et al. Modes of Action and Inhibitory Activities of New Siderophore-Beta-Lactam Conjugates That Use Specific Iron Uptake Pathways for Entry into Bacteria. Antimicrobial Agents and Chemotherapy. October 1992, Vol. 36, No. 10, pages 2166-2175, see entire document.	1-50
Y	CHAKRABORTY et al. Design and Synthesis of a Rapamycin-Based High Affinity Binding FKBP12 Ligand. Chemistry and Biology. March 1995, Vol. 2, No. 3, pages 157-161, see entire document.	1-50
Y	CRABTREE et al. Three-Part Inventions: Intracellular Signaling and Induced Proximity. Elsevier Trends Journal. November 1996, pages 418-422, see entire document.	1-50
Y	KRAMER et al. Liver-Specific Drug Targeting by Coupling to Bile Acids. The Journal of Biological Chemistry. 15 September 1992, Vol. 267, No. 26, pages 18598-18604, see entire document.	1-50
Y	LUSSOW et al. Targeting of Antihapten Antibodies to Activated T Cells via an IL-2-Hapten Conjugate Prolongs Cardiac Graft Survival. Transplantation. 27 December 1996, Vol. 62, No. 12, pages 1703-1708, see entire document.	1-50

L1 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:763899 HCAPLUS
DOCUMENT NUMBER: 132:15629
TITLE: **Bifunctional** molecules and therapies based thereon
INVENTOR(S): **Briesewitz, Roger**; Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 67 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
INT. PATENT CLASSIF.:
MAIN: A61K039-385
SECONDARY: A61K037-02; A61K047-48
CLASSIFICATION: 63-6 (Pharmaceuticals)
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9961055	A1	19991202	WO 1999-US11296	19990521
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9940937	A1	19991213	AU 1999-40937	19990521
EP 1079859	A1	20010307	EP 1999-924431	19990521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1998-86451 P 19980522
WO 1999-US11296 W 19990521
OTHER SOURCE(S): MARPAT 132:15629
ABSTRACT:
Non-naturally occurring **bifunctional** conjugates Z-L-X (Z = ligand that binds to a specific presenter protein; X = drug moiety; L = optional linker) are provided such that upon entering a cell, Z can bind to its receptor protein (if present) and the effectiveness of X is thereby enhanced or inhibited, depending on the nature of the receptor for Z. Thus, a ***bifunctional*** peptide (I) was prepd. which contained FK506 coupled to phosphotyrosyl-glutamyl-glutamyl-isoleucine (pYEEI), which binds to the SH2 domains of tyrosine kinases Fyn and Lck and to the N-terminal SH2 domain of phospholipase C.gamma. (PLC.gamma.). In the presence of FK506-binding protein 52 (FKBP52), I bound the Fyn SH2 domain with 3-fold increased affinity. This effect was reversed by FK506, and was not mimicked by FKBP12 despite the similar structure of its binding domain to that of FKBP52; the increase in affinity with FKBP52 was presumably based on favorable protein-protein interactions between the Fyn SH2 domain and FKBP52. On the other hand, formation of a FKBP12-I complex reduced the affinity of I for the PLC.gamma. SH2 domain but not for the Fyn or Lek SH2 domains, suggesting that formation of a binary complex may lead to unfavorable protein-protein interactions between the presenter protein and some targets but not other targets of the drug; therefore, formation of a complex between a **bifunctional** mol. and a presenter protein can be used to create specificity. The cell selectivity of a ***bifunctional*** conjugate may be enhanced if the formation of a binary complex reduces binding of the drug to all of its targets in a cell that contains the presenter mol.; if an organism has cells that contain the

presenter protein and other cells that do not, the cells lacking the presenter protein will be more affected by the **bifunctional** conjugate than cells expressing the presenter. Similarly, conjugation of penicillamine (an alk. phosphatase inhibitor) to p-aminosalicylic acid (a ligand for albumin) via glycine modulated the inhibitory activity of penicillamine toward 4 isoforms of alk. phosphatase in the presence of 100 .mu.M serum albumin, but not toward 8 other isoforms.

SUPPL. TERM: drug ligand conjugate structure activity; **bifunctional** peptide FK506 receptor tyrosine kinase; phospholipase C **bifunctional** peptide FK506 receptor; penicillamine aminosalicylate conjugate alk phosphatase

INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(FKBP (FK 506-binding protein), 52,000-mol.-wt., binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(FKBP-12 (FK 506-binding protein, 12,000-mol.-wt.), binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Heat-shock proteins
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(HSP 90, binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Protein motifs
(SH2 domain, binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Antimicrobial agents
Drugs
(**bifunctional** conjugates with ligands for proteins; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Coupling agents
(**bifunctional**, for drugs and protein ligands; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Albumins, biological studies
Steroid receptors
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Proteins, general, biological studies
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(binding by protein ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(cytoskeleton-assocd., binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Antibacterial agents
(dihydrofolate reductase inhibitors, modulation of

activity of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Proteins, specific or class
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(extracellular matrix-assocd., binding by protein ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Ligands
Receptors
ROLE: BAC (Biological activity or effector, except adverse); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(for proteins, **bifunctional** conjugates with drugs; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Escherichia coli
(ligand-drug **bifunctional** conjugates inhibition of, modulation of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Affinity
Chemoselectivity
(of drug-ligand **bifunctional** conjugate for drug target, enhancement of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Structure-activity relationship
(of drugs, conjugation to protein ligands effect on; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Molecular association
(of ligand-drug **bifunctional** conjugate with endogenous presenter protein and drug target; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Vitamins
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(receptors, binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: Receptors
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(vitamin, binding by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 251535-42-9
ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**bifunctional** mols. and therapies based thereon)

INDEX TERM: 59368-16-0, 2,4-Diamino-6-bromomethylpteridine 69739-34-0, tert-Butyldimethylsilyl triflate 74124-79-1, N,N'-Disuccinimidyl carbonate 104987-11-3, FK506 198472-14-9 251535-41-8
ROLE: RCT (Reactant)
(**bifunctional** mols. and therapies based thereon)

INDEX TERM: 133941-75-0P 152406-15-0P 154074-71-2P
ROLE: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(**bifunctional** mols. and therapies based thereon)

INDEX TERM: 95076-93-0, Peptidyl prolyl isomerase
ROLE: BPR (Biological process); BIOL (Biological study);

PROC (Process)
(binding by protein ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 114051-78-4 141349-87-3
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(binding of SH2 domain of, by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 251535-43-0P 251535-44-1P
ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(dihydrofolate reductase inhibition by, modulation of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 9001-78-9
ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(inhibition by ligand-drug **bifunctional** conjugates, modulation of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 9002-03-3, Dihydrofolate reductase
ROLE: BPR (Biological process); CAT (Catalyst use); BIOL (Biological study); PROC (Process); USES (Uses)
(inhibition by ligand-drug **bifunctional** conjugates, modulation of; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 9001-86-9, Phospholipase C
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(isoform .gamma., binding of SH2 domain of, by ligand-drug **bifunctional** conjugates; **bifunctional** mols. and therapies based thereon)

INDEX TERM: 225108-46-3P 251535-40-7P
ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(prepn. and binding to endogenous presenter protein, modulation of; **bifunctional** mols. and therapies based thereon)

REFERENCE COUNT: 21
REFERENCE(S):

(1) Al-Obeidi; Journal of Pharmaceutical Sciences 1990, V79(6), P500 HCAPLUS
(2) Belshaw; Proc Natl Acad Sci USA 1996, V93, P4604 HCAPLUS
(3) Briesewitz; Proc Natl Acad Sci USA 1999, V96(5), P1953 HCAPLUS
(4) Brochu; Antimicrobial Agents and Chemotherapy 1992, V36(10), P2166 HCAPLUS
(5) Cemu Bioteknik Ab; WO 9101743 A1 1991 HCAPLUS
(6) Crabtree; Trends in BS (Elsevier) 1996, V21, P418 HCAPLUS
(7) Heath; Biochem BioPhys Acta 1986, V862(1), P72 HCAPLUS
(8) Ho; Nature 1996, V382, P822 HCAPLUS
(9) Holt; Bioorganic and Medicinal Chemistry Letters 1994, V4(2), P315 HCAPLUS
(10) Karasiewicz; US 5382657 A 1995 HCAPLUS
(11) Kramer; Journal of Biological Chemistry 1992, V267(26), P18598 HCAPLUS
(12) Luengo; Bioorganic & Medicinal Chemistry Letters 1994, V4(2), P321 HCAPLUS

(13) Lussow; Transplantation 1996, V62(12), P1703 HCAPLUS
(14) Maeda; Biochemical and Biophysical Research Communications 1997, V241(2), P595 HCAPLUS
(15) President And Fellows Of HarvaRd College; WO 9847916 A1 1998 HCAPLUS
(16) Redcell Inc; WO 9510302 A1 1995 HCAPLUS
(17) The Board Of Trustees Of The Leland Stanford Junior University; WO 9418317 A1 1994 HCAPLUS
(18) The Board Of Trustees Of The Leland Stanford Junior University; WO 9502684 A1 1995 HCAPLUS
(19) Varashavsky, A; Proc Natl Acad Sci USA 1998, V95, P2094
(20) Varhavsky, A; Proc Natl Acad Sci USA 1995, V92, P3663
(21) Zunino; Eur Journal of Cancer Clin Oncol 1984, V29(3), P421

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11296

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/385, 37/02, 47/48; C07K 13/00
US CL :424/193.1; 530/351, 391.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BROCHU et al. Modes of Action and Inhibitory Activities of New Siderophore-B-Lactam Conjugates That Use Specific Iron Uptake Pathways for Entry into Bacteria. Antimicrobial Agents and Chemotherapy. October 1992, Vol. 36, No. 10, Pages 2166-2175, entire Document.	1-2, 4-6, 8, 10-15, and 46-47
Y	VARASHAVSKY, A. Codominant Interference, Antieffectors, and Multitarget Drugs. Proc. Natl. Acad. Sci. USA. March 1998, Vol. 95, Pages 2094-2099, entire document, especially Figures 1, 2, and 3.	1-15 and 46-47

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
07 AUGUST 1999	17 SEP 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T.C. McCarthy III, Ph.D. Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11296

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HEATH et al. Liposome-mediated Delivery of Pteridine Antifolates to Cells in Vitro: Potency of Methotrexate and its Alpha and Gamma Substituents. Biochem. BioPhys. Acta. 1986, Vol. 862, No. 1, pages 72-80, entire document.	1-15, and 46-47
Y	HOLT et al. Structure-Activity Studies of Synthetic FKBP Ligands as Peptidyl-Prolyl Isomerase Inhibitors, Bioorganic and Medicinal Chemistry Letters. 1994, Vol. 4, No. 2, pages 315-320, entire document, especially Tables 1-4.	1-15, and 46-47
Y	LUENGO et al. Synthesis and Structure-Activity Relationships of Macrocyclic FKBP Ligands. Bioorganic & Medicinal Chemistry Letters. 1994, Vol. 4, No. 2, pages 321-324, entire document.	1-15, and 46-47
X,P	BRIESEWITZ et al. Affinity Modulation of Small Molecule Ligands by Borrowing Endogenous Protein Surfaces. Proc. Natl. Acad. Sci. USA. 02 March 1999, Vol. 96, No. 5, pages 1953-1958, entire Document.	1-15 and 46-47
Y	WO 95/02684 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 26 January 1995, entire document.	1-15 and 46-47
Y	WO 91/01743 A1 (CEMU BIOTEKNIK AB) 21 February 1991, entire document.	1, 5, and 12
Y	CRABTREE et al. Three-Part Inventions: Intracellular Signalling and Induced Proximity. Trends in BS (Elsevier). November 1996, Vol. 21, pages 418-422, entire document.	1-15
Y	LUSSOW et al. Targeting of Antihapten Antibodies to Activated T Cells via an IL-2-Hapten Conjugate Prolongs Cardiac Graft Survival. Transplantation. 27 December 1996, Vol. 62, No. 12, pages 1703-1708, entire document.	1-15, and 46-47
Y	US 5,382,657 A (KARASIEWICZ et al.) 17 January 1995, Abstract and col. 3, lines 32-39.	1, 2, and 8
A	WO 94/18317 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 18 August 1994, entire document.	1-15 and 46-47
A	KRAMER et al. Liver-Specific Drug Targeting by Coupling to Bile Acids. Journal of Biological Chemistry. 15 September 1992, Vol. 267, No. 26, pages 18598-18604, entire document.	1-15 and 46-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11296

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BELSHAW et al. Controlling Protein Association and Subcellular Localization with a Synthetic Ligand that Induces Heterodimerization of Proteins. Proc. Natl. Acad. Sci. USA. May 1996, Vol. 93, pages 4604-4607, entire document.	1-15 and 46-47
A	HO et al. Dimeric Lignads Define a Role for Transcriptional Activation Domains in Reinitiation. Nature. 29 August 1996, Vol. 382, Pages 822-826, entire document.	1-15 and 46-47
A	AL-OBEIDI et al. Synthesis and Actions of a Melanotropin Conjugate, Ac-[Nle, Glu(gamma-4'-hydroxtanilide)5, D-Phe]alpha-MSH4-10-NH2, on Melanocytes and Melanoma Cells in Vitro. Jounral of Pharmaceutical Sciences. June 1990, Vol. 79, No. 6, pages 500-504, entire document.	1-15 and 46-47
A	MAEDA et al. Amino Acids and Peptides XXXII: A Bifunctional Poly(Ethylene Glycol) Hybrid of Fibronectin-Related Peptides. Biochemical and Biophysical Research Communications. 1997, Vol. 241, No. 2, pages 595-598, entire document.	1-15 and 46-47
A	ZUNINO et al. Comparison of Antitumor Efects of Daunorubicin Covalently Linked to Poly-L-Amino Acid Carriers, Eur. Journal of Cancer Clin. Oncol., 1984, Vol. 29, No. 3, pages 421-425, entire document.	1-15 and 46-47
A,P	WO 98/47916 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 29 October 1998, entire document.	1-15 and 46-47
A	WO 95/10302 A1 (REDCELL, INC.) 20 April 1995, entire document.	1-15 and 46-47
Y	VARHAVSKY, A. Codominance and Toxins: A Path to Drugs of Nearly Unlimited Selectivity. Proc. Natl. Acad. Sci. USA. April 1995, Vol. 92, pages 3663-3667, entire document.	1-15 and 46-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11296

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/93.21, 193.1, 133.1, 280.1; 435/183; 514/6; 525/408; 530/317, 351, 391.7, 385; A61K 39/395, 37/02; C07K 16/28 , 16/46, 14/00; C12N 15/62, 15/00, 5/00. 5/06

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE (STN: CAPLUS, BIOSIS, SCISEARCH), KEYWORDS SEARCHED: CONJUGATE, CONJUGATES, LINKED, BONDED, BOUND, ATTACHED, PROTEIN, PEPTIDE, LIGAND, AFFINITY, FK-506, RAPAMYCIN, CYCLOSPORIN A, FKBP, FK-506 BINDING PROTEIN, TOXICITY, CYTOTOXICITY, IMMUNOSUPPRESIVE, ANTIBODY, ANTIGEN, HAPTEN, RECEPTOR, EXTRACELLULAR, INTRACELLULAR, ALBUMIN, BACTERIA, ANTIMICROBIAL

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-15 and 46-47, drawn to a drug-ligand conjugate and a kit.
Group II, claim(s) 16-22, drawn to a method of producing a binary complex in host.
Group III, claim(s) 23-28, drawn to a method for producing a tripartite complex in host.
Group IV, claim(s)29-32, drawn to a method of producing an intracellular tripartite complex in host.
Group V, claim(s) 33-34, drawn to a method of producing a binary complex in host.
Group VI, claim(s) 35-37, drawn to a method for enhancing the selectivity of a drug.
Group VII, claim(s)38-44, drawn to a method for administering a drug.
Group VIII, claim(s) 45, drawn to a method of identifying potentially active drugs using a combinatorial library.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the invention of group I (i.e. the sepcial technical feature) lacks an inventive step, and therefore lacks novelty. Heath et al. (1987), and Roskowsky teach that dihydrofolate reductase-inhibting drugs are potent and toxic antimicrobial drugs. Holt et al. (1994) teach that small molecules can be used as analogues to immunosuppresive proteins such as FK-506. Varashavsky (1995) and Varashavsky (1998) (a) teach that by conjugating low-molecullar weight toxic drugs to low molecular weight ligands, an active antibiotic-ligand conjugate can be formed and made selectively toxic for cells that lack a particular protein, and (b) therefore provide motivation to combine the teachings of Holt et al. (1994) with those of Heath et al. (1994) and Rosowsky (1985). Furthermore, Crabtree et al. (WO 94/18317 A1) disclose low-molecular weight, bifunctional drugs that fit all of the limitations of the applicants' claims 1-15.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11296

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-15, and 46-47

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

WHAT IS CLAIMED IS:

1. A non-naturally occurring bifunctional molecule of less than about 5000 daltons consisting of a drug moiety and a presenter protein ligand, wherein said drug moiety and said presenter protein ligand are optionally joined by a linking group and said drug moiety has enhanced activity as compared to a free drug control.

2. The bifunctional molecule according to Claim 1, wherein said bifunctional molecule comprises a linking group.

3. The bifunctional molecule according to Claim 1, wherein said drug moiety exhibits at least one of enhanced affinity, specificity or selectivity for its target as compared to a free drug control.

4. The bifunctional molecule according to Claim 1, wherein said drug moiety binds to a protein target.

5. The bifunctional molecule according to Claim 1, wherein said presenter protein ligand binds to an extracellular protein.

6. The bifunctional molecule according to Claim 1, wherein said presenter protein ligand binds to an intracellular protein.

7. The bifunctional molecule according to Claim 6, wherein said presenter protein ligand is a ligand for a peptidyl prolyl isomerase.

8. A synthetic bifunctional molecule of less than about 5000 daltons of the formula:

Z-L-X

wherein:

X is a drug moiety;

L is a bond or a linking group; and

Z is a ligand for an endogenous presenter protein ligand;

wherein X and Z are different and said drug moiety has enhanced activity as compared to a free drug control.

9. The bifunctional molecule according to Claim 8, wherein said drug moiety exhibits at least one of enhanced affinity, specificity or selectivity for its target as compared to a free drug control.

10. The bifunctional molecule according to Claim 8, wherein said drug moiety has a molecular weight of from about 50 to 2000 D.

11. The bifunctional molecule according to Claim 8, wherein said drug moiety binds to a protein target.

12. The bifunctional molecule according to Claim 8, wherein said presenter protein ligand binds to an extracellular protein.

13. The bifunctional molecule according to Claim 8, wherein said presenter protein ligand binds to an intracellular protein.

14. The bifunctional molecule according to Claim 13, wherein said presenter protein ligand is a ligand for a peptidyl prolyl isomerase.

15. The bifunctional molecule according to Claim 8, wherein said presenter protein ligand has substantially no pharmacologic activity apart from binding to a presenter protein ligand.

16. A method for producing a binary complex in a host, said method comprising:

administering to said host an effective amount of a bifunctional molecule of less than about 5000 daltons consisting of a drug moiety and a ligand for a presenter protein endogenous to said host, wherein said drug moiety and ligand are optionally joined by a linking group;

whereby a binary complex comprising said bifunctional molecule and presenter protein is produced that exhibits enhanced drug activity as compared to a free drug control.

17. The method according to Claim 16, wherein said enhanced drug activity comprises at least one of enhanced affinity, specificity or selectivity of said drug moiety for a target of said drug moiety.

27. The method according to Claim 23, wherein said endogenous presenter protein is selected from the group consisting of: peptidyl-prolyl isomerases, Hsp90, steroid hormone receptors, cytoskeletal proteins, albumin and vitamin receptors.

28. The method according to Claim 23, wherein said bifunctional molecule is administered as a pharmaceutical preparation.

29. A method for producing an intracellular tripartite complex in a mammalian host, said method comprising:

administering to said mammalian host an effective amount of a bifunctional molecule comprising a drug moiety and an endogenous presenter protein ligand, wherein the target of said drug and said endogenous presenter protein are intracellular proteins;

whereby said bifunctional molecule binds to said drug target and endogenous presenter protein to intracellularly produce said tripartite complex, wherein said tripartite complex is characterized by the presence of presenter protein target binding interactions which result in enhanced drug activity as compared to a free drug control.

30. The method according to Claim 29, wherein said target protein is an enzyme

31. The method according to Claim 29, wherein said endogenous presenter protein is selected from the group consisting of: peptidyl prolyl isomerases, Hsp90, steroid hormone receptors and cytoskeletal proteins.

32. The method according to Claim 31, wherein said endogenous presenter protein is a peptidyl prolyl isomerase.

33. A method for producing a binary complex in a host, said method comprising:

administering to said host an effective amount of a bifunctional molecule comprising a drug moiety and a ligand for a presenter protein endogenous to said host;

whereby a binary complex comprising said bifunctional molecule and presenter protein is produced that exhibits enhanced specificity for a target of said drug moiety target as compared to a free drug control.

34. The method according to Claim 33, wherein said ligand for a presenter protein is a peptidyl prolyl isomerase.

35. A method for enhancing the selectivity of a drug for a target in a first cell as compared to a second cell, said method comprising:

contacting said first and second cells with a bifunctional molecule comprising said drug and a ligand for a presenter protein present in said second cell but not in said first cell;

whereby a binary complex comprising said bifunctional molecule and presenter protein is produced in said second cell but not said first cell.

36. The method according to Claim 35, wherein said drug moiety is an antimicrobial agent.

37. The method according to Claim 35, wherein said ligand is a peptidyl prolyl isomerase ligand.

38. In a method of administering a drug to a host in need of said drug, the improvement comprising:

administering to said host an effective amount of a bifunctional molecule of less than about 5000 daltons consisting of said drug or a fragment thereof covalently linked, either directly or through an optional linking group, to a ligand for a presenter protein endogenous to said host.

39. The method according to Claim 38, wherein said host is a mammalian host.

40. The method according to Claim 39, wherein said mammalian host is human.

41. The method according to Claim 38, wherein said drug is a small molecule.

42. The method according to Claim 38, wherein said drug binds to an extracellular target.

43. The method according to Claim 38, wherein said drug binds to an intracellular target.

44. The method according to Claim 43, wherein said presenter protein ligand is a peptidyl prolyl isomerase.

45. A method of making a bifunctional molecule comprising a drug moiety that exhibits at least one of enhanced affinity, specificity or selectivity as compared to the corresponding free drug, said method comprising:

identifying a drug moiety;

preparing a library of bifunctional molecules comprising said drug moiety and a ligand for a presenter protein, wherein each bifunctional molecule shares a common ligand and drug moiety separated by a variable linking; and

screening said library to identify those members having at least one of enhanced affinity, specificity or selectivity as compared to the corresponding free drug.

46. A pharmaceutical preparation comprising a bifunctional molecule according to Claim 1.

47. A kit comprising the pharmaceutical preparation according to Claim 46 and instructions for use in a therapeutic method.

Trying 3106016892...Open

Welcome to STN International! Enter x:x
LOGINID:ssspta1644pnh
PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Dec 17	The CA Lexicon available in the CAPLUS and CA files
NEWS 3	Feb 06	Engineering Information Encompass files have new names
NEWS 4	Feb 16	TOXLINE no longer being updated
NEWS 5	Apr 23	Search Derwent WPINDEX by chemical structure
NEWS 6	Apr 23	PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
NEWS 7	May 07	DGENE Reload
NEWS 8	Jun 20	Published patent applications (A1) are now in USPATFULL
NEWS 9	JUL 13	New SDI alert frequency now available in Derwent's DWPI and DPCI
NEWS 10	Aug 23	In-process records and more frequent updates now in MEDLINE
NEWS 11	Aug 23	PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
NEWS 12	Aug 23	Adis Newsletters (ADISNEWS) now available on STN
NEWS 13	Sep 17	IMSworld Pharmaceutical Company Directory name change to PHARMASEARCH

NEWS EXPRESS	August 15 CURRENT WINDOWS VERSION IS V6.0c, CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP), AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 18:45:01 ON 18 SEP 2001

=> file medline embase biosis scisearch caplus

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.15	0.15

FILE 'MEDLINE' ENTERED AT 18:45:15 ON 18 SEP 2001

FILE 'EMBASE' ENTERED AT 18:45:15 ON 18 SEP 2001

FILE 'BIOSIS' ENTERED AT 18:45:15 ON 18 SEP 2001

FILE 'SCISEARCH' ENTERED AT 18:45:15 ON 18 SEP 2001

FILE 'CAPLUS' ENTERED AT 18:45:15 ON 18 SEP 2001

=> s method

4 FILES SEARCHED...
L1 9147254 METHOD

=> s l1 and biodistribution

L2 5475 L1 AND BIODISTRIBUTION

=> s l2 and "less than about 5000 daltons"

L3 0 L2 AND "LESS THAN ABOUT 5000 DALTONS"

=> s l2 and bifunctional molecule

L4 1 L2 AND BIFUNCTIONAL MOLECULE

=> d l4 cbib abs

L4 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
2001:380414 Document No. 134:371812 Targeted **bifunctional molecules** and therapies based thereon. Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted **bifunctional mols**. and **methods** for their use are provided. The subject targeted **bifunctional mols**. are conjugates of a drug moiety and a targeting moiety, where these two moieties are optionally joined by a linking group. The **bifunctional mols**. are further characterized in that they exhibit a modulated **biodistribution** upon administration to a host as compared to a free drug control. The subject targeted **bifunctional mols**. find use in a variety of therapeutic applications. For example, a **bifunctional mol**. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this **bifunctional mol**. enters the human circulation, it is bound by albumin which keeps the drug of interest in the

extracellular environment.

=> d his

(FILE 'HOME' ENTERED AT 18:45:01 ON 18 SEP 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 18:45:15 ON 18 SEP 2001

L1 9147254 S METHOD
L2 5475 S L1 AND BIODISTRIBUTION
L3 0 S L2 AND "LESS THAN ABOUT 5000 DALTONS"
L4 1 S L2 AND BIFUNCTIONAL MOLECULE

=> s l2 and molecule

L5 402 L2 AND MOLECULE

=> s l5 and biodistribution

L6 402 L5 AND BIODISTRIBUTION

=> s l6 and "5000 daltons"

L7 0 L6 AND "5000 DALTONS"

=> s l5 and "5000"

L8 1 L5 AND "5000"

=> d l8 cbib abs

L8 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
1994:465435 Document No. 121:65435 **Biodistribution** and antitumor effect of adriamycin encapsulated in long-circulating liposomes containing amphipathic polyethylene glycol or ganglioside GM1. Maruyama, Kazuo; Okamoto, Aki; Ishida, Osamu; Kojima, Shuji; Suginaka, Akinori; Huang, Leaf; Iwatsuru, Motoharu (Fac. Pharm. Sci., Teikyo Univ., Sagamiko, 199-01, Japan). J. Liposome Res. 701-23 (English) 1994. CODEN: JLREE7. ISSN: 0898-2104.

AB **Biodistribution** and antitumor effect of adriamycin (ADM) encapsulated in liposomes with reduced uptake by reticuloendothelial system (RES) and prolonged circulation time were investigated in mice. Two different types of long-circulating liposomes, ganglioside GM1 (GM1)/distearoylphosphatidylcholine (DSPC) /cholesterol (CH) (0.13:1:1) and amphipathic polyethylene glycol (PEG)/DSPC/CH (0.13:1:1) were used. They were sized to 180-200 nm in mean diam. In the case of amphipathic PEG, distearoylphosphatidylethanolamine (DSPE) derivs. of PEG with various **mol**. wt. (1000-12,000 in mean **mol**. wt.) were used. ADM was encapsulated by transmembrane pH gradient **method**. GM1/DSPC/CH liposome entrapped ADM with over 95% in trapping efficiency and its drug retention after incubation with 20% serum in PBS (pH 7.4) for 24 h was 92%. Similar results were obtained with liposomes contg. amphipathic PEG with av. **mol**. wt. of 1000, 2000 and 3000. However, the liposomes contg. high **mol**. wt. PEG such as **5000** and 12,000 showed decreased trapping efficiency such as 82% and 60%, resp. ADM-GM1/DSPC/CH liposomes and ADM-PEG/DSPC/CH liposomes showed low uptake by RES and high blood concn. at 6 h after i.v. injection, compared with ADM-DSPC/CH liposomes. ADM-PEG1000/DSPC/CH

liposomes showed the highest concn. in blood among all PEG-liposomes. ADM
concns. assocd. with RES (liver and spleen) of ADM-PEG1000/DSPC/CH and ADM-GM1/DSPC/CH were lower than that of ADM-DSPC/CH over the entire 24-h period. The antitumor efficacy of liposomal ADM was estd. in the L1210 leukemia tumor-bearing mice. Free ADM or liposomal ADM was injected at a dose of 2.0 or 5.0 mg/kg one day after tumor cell inoculation. When treated at a dose of 2.0 mg/kg, liposomal ADM displayed antitumor effect similar to that of free drug. The administration of ADM-GM1/DSPC/CH or ADM-PEG1000/DSPC/CH liposomes at a dose of 5.0 mg/kg to tumor bearing mice
induced prolonged survival with 100% survival rate for at least 60 days after treatment.

=> s briesewitz r?/au or Crabtree g?/au or wandless t?/au

L9 2181 BRIESEWITZ R?/AU OR CRABTREE G?/AU OR WANDLESS T?/AU

=> s l9 and bifunctional molecule

L10 13 L9 AND BIFUNCTIONAL MOLECULE

=> dup remove l10

PROCESSING COMPLETED FOR L10
L11 7 DUP REMOVE L10 (6 DUPLICATES REMOVED)

=> d l11 1-7 cbib abs

L11 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2001 ACS
2001:380414 Document No. 134:371812 Targeted **bifunctional molecules** and therapies based thereon. **Briesewitz, Roger ; Crabtree, Gerald R.; Wandless, Thomas** (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted **bifunctional mols**. and methods for their use are provided. The subject targeted **bifunctional mols**. are conjugates of a drug moiety and a targeting moiety, where these two moieties are optionally joined by a linking group. The **bifunctional mols**. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted **bifunctional mols**. find use in a variety of therapeutic applications. For example, a **bifunctional mol**. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this **bifunctional mol**. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

L11 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2001 ACS
2001:380323 Document No. 134:361342 Drug conjugates with pharmacokinetic modulating moieties, and therapies based thereon. **Briesewitz,**

Roger; **Crabtree, Gerald R.**; **Wandless, Thomas** (The Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035748 A1 20010525, 38 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31701 20001117. PRIORITY: US 1999-PV166633 19991119.

AB **Bifunctional mols**. and methods for their use are provided. The **bifunctional mols**. of the invention are conjugates of a drug moiety and a pharmacokinetic modulating moiety, optionally joined by a linking group. The **bifunctional mols**. are further characterized in that they exhibit at least one modulated pharmacokinetic property upon administration to a host as compared to a free drug control. The subject **bifunctional mols**. find use in a variety of therapeutic applications.

L11 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2001 ACS

2001:203206 Cell-selective detoxification by borrowing endogenous proteins. Lin, Yun-Ming; Braun, Patrick D.; Ray, Gregory T.; **Wandless, Thomas J.** (Department of Chemistry, Stanford University, Stanford, CA, 94305, USA). Abstr. Pap. - Am. Chem. Soc., 221st, ORGN-698 (English) 2001. CODEN: ACSRAL. ISSN: 0065-7727. Publisher: American Chemical Society.

AB Undesired side effects to mammalian cells are often encountered in enzyme inhibitor-based chemotherapy. Potential cell-selective detoxification could be achieved by converting therapeutic agents into **bifunctional mols**. capable of recruiting endogenous proteins unique to mammalian cells as their protecting groups. Covalent attachment of a dihydrofolate reductase (DHFR) inhibitor to a synthetic ligand (SLF) for FK506 binding proteins (FKBP) affords a **bifunctional mol**. which retains efficacy against DHFR. However, this efficacy is diminished or lost in the presence of FKBP, presumably due to unfavorable protein-protein interactions in the trimeric complex. Borrowing endogenous proteins by **bifunctional mols**. to achieve cell-selective detoxification could provide a novel strategy for drug development. The synthesis and biochem. assay of a **bifunctional mol**. for DHFR and FKBP will be presented.

L11 ANSWER 4 OF 7 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1

2000439781 EMBASE Mechanistic studies of affinity modulation. Rosen M.K.; Amos C.D.; **Wandless T.J.**. T.J. Wandless, Department of Chemistry, Stanford University, Stanford, CA 94305, United States. wandless@stanford.edu. Journal of the American Chemical Society 122/48 (11979-11982) 6 Dec 2000.
Refs: 23.
ISSN: 0002-7863. CODEN: JACSAT. Pub. Country: United States. Language: English. Summary Language: English.

AB A synthetic ligand for the protein FKBP12 was covalently linked to a peptide ligand (pYEEI) for the Fyn SH2 protein to create a **bifunctional molecule** called SLFpYEEI. This **bifunctional molecule** can simultaneously bind both proteins to form a trimeric complex. When SLFpYEEI is precomplexed with FKBP12, the peptide ligand binds 6-fold more weakly to the Fyn SH2 domain than SLFpYEEI alone. Isotope-edited NMR spectroscopy was used to investigate the molecular basis for the observed reduction in affinity. The results suggest that interactions between the pYEEI peptide and FKBP12

may play a significant role in diminishing the affinity of SLFpYEEI for the Fyn SH2 domain.

L11 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2001 ACS
2000:332410 Improving protein-ligand interactions.. **Wandless, Thomas J.** (Department of Chemistry, Stanford University, Stanford, CA, 94305-5080, USA). Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000, ORGN-283. American Chemical Society: Washington, D. C. (English) 2000. CODEN: 69CLAC.
AB One strategy to improve the binding between a small mol. ligand and its protein receptor is to increase the surface area at the binding interface.
Addnl. interactions may contribute, either pos. or neg., to the overall free energy of binding. We have developed a potentially general method to
improve or diminish the binding affinity between a ligand and its protein target. The X-ray structures of two different small mols. bound to two different protein targets were analyzed. Synthetic chem. was used to link
the two compds., thus creating a bifunctional ligand for both proteins. When tethered with the proper geometry, the **bifunctional mol**. allows both protein receptors to bind simultaneously, and addnl. protein-protein interactions are created that contribute pos. or neg. to the overall stability of the trimeric complex.

L11 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2001 ACS
1999:763899 Document No. 132:15629 **Bifunctional molecules** and therapies based thereon. **Briesewitz, Roger**; **Crabtree, Gerald R.**; **Wandless, Thomas**; Ray, Gregory Thomas; Vogel, Kurt William (The Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 9961055 A1 19991202, 67 pp.
DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH,
CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US11296 19990521. PRIORITY: US 1998-86451 19980522.
AB Non-naturally occurring bifunctional conjugates Z-L-X (Z = ligand that binds to a specific presenter protein; X = drug moiety; L = optional linker) are provided such that upon entering a cell, Z can bind to its receptor protein (if present) and the effectiveness of X is thereby enhanced or inhibited, depending on the nature of the receptor for Z. Thus, a bifunctional peptide (I) was prepd. which contained FK506 coupled to phosphotyrosyl-glutamyl-glutamyl-isoleucine (pYEEI), which binds to the
SH2 domains of tyrosine kinases Fyn and Lck and to the N-terminal SH2 domain of phospholipase C.gamma. (PLC.gamma.). In the presence of FK506-binding protein 52 (FKBP52), I bound the Fyn SH2 domain with 3-fold increased affinity. This effect was reversed by FK506, and was not mimicked by FKBP12 despite the similar structure of its binding domain to that of FKBP52; the increase in affinity with FKBP52 was presumably based on favorable protein-protein interactions between the Fyn SH2 domain and FKBP52. On the other hand, formation of a FKBP12-I complex reduced the affinity of I for the PLC.gamma. SH2 domain but not for the Fyn or Lek SH2
domains, suggesting that formation of a binary complex may lead to unfavorable protein-protein interactions between the presenter protein and
some targets but not other targets of the drug; therefore, formation of a complex between a **bifunctional mol**. and a presenter

protein can be used to create specificity. The cell selectivity of a bifunctional conjugate may be enhanced if the formation of a binary complex reduces binding of the drug to all of its targets in a cell that contains the presenter mol.; if an organism has cells that contain the presenter protein and other cells that do not, the cells lacking the presenter protein will be more affected by the bifunctional conjugate than cells expressing the presenter. Similarly, conjugation of penicillamine (an alk. phosphatase inhibitor) to p-aminosalicylic acid (a ligand for albumin) via glycine modulated the inhibitory activity of penicillamine toward 4 isoforms of alk. phosphatase in the presence of 100 .mu.M serum albumin, but not toward 8 other isoforms.

L11 ANSWER 7 OF 7 MEDLINE DUPLICATE 2
1999162539 Document Number: 99162539. PubMed ID: 10051576. Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces. **Briesewitz R**; Ray G T; **Wandless T J**; **Crabtree G R**. (Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Mar 2) 96 (5) 1953-8. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A general strategy is described for improving the binding properties of small-molecule ligands to protein targets. A **bifunctional molecule** is created by chemically linking a ligand of interest to another small molecule that binds tightly to a second protein. When the ligand of interest is presented to the target protein by the second protein, additional protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biologically active compounds.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	53.73	53.88

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-4.12	-4.12

* *

Dear valued customer,

Your feedback is important to us. Would you kindly take a moment to complete our survey? This survey will only take about 5-10 minutes to

complete. Your responses will be kept confidential and will help us improve STN Express with Discover! for your future use. Please click on the following link to access the survey.

http://www.cas.org/ONLINE/STN/ExpressSurveyForm.html?LOGINID=SSSPTA1644PNH

* *

STN INTERNATIONAL LOGOFF AT 18:50:36 ON 18 SEP 2001